MMS Sensitivity of All Amino Acid-Requiring Mutants in *Aspergillus* and Its Suppression by Mutations in a Single Gene

Etta Käfer

Department of Biology, McGill University, Montreal H3A 1B1, Canada

Manuscript received September 22, 1986

Accepted December 31, 1986

ABSTRACT

All available amino acid-requiring mutants of *Aspergillus nidulans* were found to be hypersensitive to MMS (methyl methanesulfonate) to various degrees. On MMS media, secondary mutations could be selected which suppress this MMS sensitivity but do not affect the requirement. Many such mutations were analyzed and found to be alleles of one gene, *smsA* (=suppressor of MMS sensitivity), which mapped distal on the right arm of chromosome V. This gene is more likely to be involved in general regulation of amino acid biosynthesis than MMS uptake, since a variety of pathway interactions were clearly modified by *smsA* suppressors in the absence of MMS.

THE finding that many DNA repair-deficient *uvr* mutations of *Aspergillus* are sensitive to MMS greatly facilitated their genetic mapping (KÄFER and MAYOR 1986). Unexpectedly, such tests revealed that certain mapping strains grew almost as poorly on MMS media as some *uvr* mutants. Analysis of haploid segregants from heterozygous diploids indicated that in all cases this hypersensitivity was associated with mutations which require amino acids (AA) for growth, while no other markers influenced growth on MMS media. Similar tests of various AA-requiring mutants of *Neurospora* did not identify any cases of hypersensitivity to MMS, and none had been observed when various MMS-sensitive *mus* mutants were crossed to such markers for genetic mapping (KÄFER and PERLMUTTER 1980). The same must be the case for yeast, since AA-requiring mutations are used as markers in many genetic tests of MMS-sensitive mutants without problems (e.g., for tests of recombination) (BORAM and ROMAN 1976; PRAKASH *et al.* 1980). On the other hand, an analogous finding for strains of yeast which require *aromatic* AA has recently been published (MEADE and MANNEY 1983). Apparently all such mutations are associated with hypersensitivity to phenethyl alcohol (PEA) which is believed to inhibit growth by affecting the cell membrane. For the AA mutations of *Aspergillus*, a plausible hypothesis might be that such strains take up MMS more rapidly than wild type. However, the results of the genetic analysis presented here indicate that this explanation is unlikely to be correct. Mutations suppressing the MMS sensitivity of AA mutants were readily isolated and these affected a variety of growth responses on media which did not contain MMS.

MATERIALS AND METHODS

Strains: Table 1 lists the analyzed AA-requiring mutations and gives genotypes for the strains used in the first test

crosses (deposited at and available from FGSC, Fungal Genetics Stock Center, Department of Microbiology, The University of Kansas, Kansas City, Kansas 66103). All other AA-requiring strains which were available from FGSC were tested for MMS sensitivity without further crossing. For analysis of pathway interactions, the *nicB* strain was FGSC 122 (*riboA1* γ A2;*nicB8*) and the *fpaA* strain was FGSC 387 (*fpaA91* *proA11* γ A2;*pyroA4*).

Media and genetic procedures: Standard *Aspergillus* media and methods for genetic analysis were used as originally devised by PONTECORVO *et al.* (1953) and up-dated in BARRATT, JOHNSON and OGATA (1965) and SCOTT and KÄFER (1982). Complete medium (CM) was used in all cases for plating of ascospores from crosses, for purification of haploid segregants from diploids by streaking out, and for master plates. To test for resistance markers or sensitivity to MMS, the latter were needle-replicated to CM containing suitable concentrations of inhibitor. Requirements were tested by replicating to supplemented minimal media (MM) lacking a single growth factor and to fully supplemented MM as controls [further details of procedures can be found in the "Appendix" to KÄFER (1977) which is available on request]. For the mapping of suppressor mutations to specific chromosomes, heterozygous diploids were used (KÄFER 1958) and these were haploidized by benlate (final concentration in CM 0.6–0.7 μ g/ml) (HASTIE 1970). To test progeny from crosses heterozygous for *fpaA*, which confers resistance to the analogue *p*-fluorophenylalanine (pfp), 0.3–0.35 g of pfp was added per liter complete medium.

Tests for sensitivity to MMS: AA-requiring and control strains were tested for growth on MMS media by transferring mass inocula of conidia to CM containing MMS (0.005–0.03%) (KÄFER and MAYOR 1986). For survival curves, conidia were either plated to CM containing these same concentrations of MMS, or treated for 2 hr (at 30°) in buffer with tenfold higher concentrations of MMS and diluted into 2–5% Na-thiosulfate to stop the reaction before plating onto CM media. The resulting colonies were counted after 3 days of incubation at 37° (longer incubation may result in additional colonies, but after 4–5 days MMS becomes heat-inactivated at 37° and counts were often erratic).

TABLE 1

Genotypes and FGSC numbers of original AA-requiring strains used, and identified suppressors of MMS sensitivity in these and derived strains^a

AA mutant	No.	MMS-sensitive strains Genotype ^b	Non-MMS-sensitive mutations ^a			
			<i>sms</i>		Other types	
			<i>smsA</i> alleles	Other genes or unknown	Suppressors	Revertants
ileA3	505	pabaA yA; AcrA wA ileA	<i>smsA</i> 7			
luA1	406	luA yA	A72			
lysA1	638	yA; lysA	A42	<i>sms</i> -41		
lysB5	360	AcrA; lysB; chaA	A51, 52			
lysD18	294	biA; sB; lysD		<i>sms</i> -62		
D20	645	biA; AcrA; lysD T(III; VII); chaA	A61			
lysE13	296	biA; sB; lysE		<i>sms</i> -91		
lysF88	293	lysF biA; sB	A82, 83, 84	<i>sms</i> -81		
methB3	272	biA; methB	A31, 32			methB-R5
methG1	219	biA; methG	A11		smt-203	
methH2	644	riboA yA; methH dilA	A21, 22		smt-206	methH-R4
phenA2	498	biA; phenA	A1, 2			
phenB6	394	biA; phenB	A6			
proA1	437	proA; wA; nicA; malA		<i>sms</i> B3		
A2	364	suAadE proA yA adE; AcrA wA		<i>sms</i> -94, 95		
proB94	386	proB (veA ⁺)				
trpA69	235	pabaA yA; trpA				trpA-R1
trpD432	238	pabaA yA; trpD	A20			

^a Mutations which reduced MMS sensitivity were selected on MMS media and called *sms* (*suppressor of MMS sensitivity*) if they did not affect the AA requirement and were not closely linked to the original AA mutation.

^b All strains except 386 carry *veA1* (velvet) which increases conidiation.

RESULTS

Analysis of MMS-sensitivity in strains with different AA requirements: Table 1 lists 18 AA-requiring mutants which showed reduced growth on MMS media (Figure 1a). These were crossed to "standard" strains (=backcrossed strains carrying a color marker and 1–2 mutations causing vitamin requirements). In all cases, AA requirement and MMS sensitivity cosegregated (a total of 1363 progeny tested, 48% of which were AA requiring and MMS sensitive). All other AA mutants available were tested for growth on MMS and also found to be hypersensitive (12 tested, including cases requiring histidine or arginine; Figure 2a).

The level of inhibition during growth on CM plus MMS varied considerably. For example, *meth* mutations caused a smaller reduction in growth than *phenA* (Figure 1a) and such *meth* strains showed practically normal levels of survival when conidia were plated (*methB* in Figure 2a). Most other AA mutants exhibited much higher inhibition on MMS media, except for some leaky alleles which were generally less sensitive (e.g., *proA1*, Figure 1a, or *phenB*, Figure 2a).

An apparent exception was the original *lysD20* strain (FGSC 395, Table 1). It grew like the wild type on MMS media, while *lysD18* (FGSC 294) was as sensitive as other *lys* strains (Figure 2b). However, when *lysD20* was crossed to a standard strain, *lysD* segregants were recovered which were as sensitive as

FIGURE 1.—Effects of *smsA* on the growth response of AA mutants on different test media (2 days at 37°). Gene symbols indicate requirements: *meth* = methionine, *phen* = phenylalanine, *pro* = proline, *nic* = nicotinic acid. Phen, Nic and Lys (lysine) indicate the corresponding supplements in minimal media (MM). (a) Variation of MMS sensitivity of different AA-requiring strains in the presence and absence of *smsA* (see also Figure 2a). Test media as labeled: Control (MM + all AA); CM + MMS contained 0.02% MMS which slightly retards growth of wild type. (b) *nicB-phenA* interaction modified by *smsA* in the absence of MMS. Test media: Control (MM + Phen and Nic); MM + Nic, lacking Phen; MM + Phen, lacking Nic. Improved growth on CM + 0.015% MMS demonstrates presence of *smsA*.

FIGURE 2.—Survival of conidia from different AA-requiring and control strains, plated onto CM + MMS (0.01 to 0.03%). (a) Low MMS sensitivity of *methB* and leaky *phenB* strains compared to other AA mutants. (b) Effect of *smsA* in *lys* strains, and survival of original *lysD* strain shown to carry a suppressor ("sup").

the *lysD18* strains (one of these has been deposited at FGSC, no. 645, Table 1). Apparently an unlinked secondary mutation is present in the original *lysD20* strain which suppresses the MMS sensitivity of *lysD20* (Table 2).

Direct isolation of MMS sensitive AA-requiring mutations: MMS-sensitive mutations have been isolated recently after UV- and γ -irradiation of *Aspergillus* conidia (KAFFER and MAYOR 1986). When these mutants were tested for requirements a considerable number of AA-requiring types were identified (eight cases requiring lysine, arginine or methionine out of 21 mutations from a total of 8500 mutagenized isolates).

Isolation and genetic analysis of suppressors of MMS sensitivity: When MMS-sensitive mutants are inoculated by conidial transfers to inhibitory concentrations of MMS and incubated, irregular sector-like growth is often observed for some inocula but not for others. Usually such "sectors" are as sensitive as the

original strain and represent conidia which survived until MMS became heat inactivated. However, in the case of AA-requiring mutants, a fraction of the sectors from MMS media showed permanent loss of MMS sensitivity, apparently as a result of suppressor mutations.

Specific suppressors of MMS sensitivity of AA-requiring strains: For the genetic analysis of such suppressors, crosses to three types of strains were routinely set up: (1) to a standard strain as a check for segregation and possible linkage of the suppressor to the AA mutation in question; (2) to another AA-requiring strain to test for "cross-suppression" of the hypersensitivity to MMS, and (3) to other suppressor strains to check for allelism. In addition, a number of suppressor strains were combined with mitotic mapping strains or with each other in heterozygous diploids, to map suppressors to different chromosomes which would rule out allelism. Two of these mapping diploids were homozygous for an AA-requiring mu-

TABLE 2

Segregation of suppressors of MMS sensitivity (*smsA*) among AA-requiring progeny from heterozygous crosses between nonhypersensitive AA strains (as listed) and standard vitamin-requiring strains

Nonhypersensitive isolates		Frequency of AA-requiring progeny			Total tested (no.)
AA-requiring mutant	Suppressor of MMS-sensitivity	Total (%)	MMS sensitive (%)	Not sensitive (%)	
LysD	<i>smsA61</i>	36	18	18	(56)
lysB	<i>smsA51</i>	38	19	19	(93)
lysA	<i>smsA42</i>	35	13	22	(100)
lysF	<i>smsA82</i>	52	30	22	(91)
phenA	<i>smsA1</i>	54	31	23	(101)
	<i>smsA2</i>	45	26	19	(94)
methH	<i>smsA22</i>	48	21	27	(186)
Average frequency (Total No.)		45.1 (325)	22.6 (163)	22.5 (162)	(721)

tation and both showed sensitivity to MMS. This indicates that the suppressor mutations (which were heterozygous) are recessive.

1. Results of the first type of crosses are shown in Table 2. They demonstrate that in each case mutations which suppress the MMS-sensitivity were segregating independently and therefore were unlinked to the AA mutations (which are present in about half of the progeny; a few exceptional cases are discussed below).

2. Seven crosses of the second type were analyzed by testing about 100 progeny for AA requirements and MMS sensitivity. In these intercrosses of two different AA mutations, the suppressor which had been selected in one of the parental strains also suppressed the hypersensitivity of the other AA mutation.

3. For many other suppressor isolates tests of "cross-suppression" were combined with tests forallelism in intercrosses, and six pairs were tested for complementation in diploids heterozygous for two different AA mutants as well as their suppressors. In addition, eight suppressors of different AA strains (including that found in the original *lysD20* strain) were mapped by testing mitotic haploid segregants from heterozygous diploids. All suppressors were found to be located on chromosome V (total haploid segregants tested: 946), and all six pairs of suppressors in diploids were found to be noncomplementing. These mutations therefore are alleles of the same gene, *smsA* (=suppressor of MMS sensitivity). Eleven further alleles were identified (as listed in Table 1) in intercrosses between unlinked AA mutations in which one parent carried a mapped *smsA* mutation and the other an uncharacterized suppressor (a total of 2740 progeny tested, 74.4% were AA requiring but none were hypersensitive to MMS).

Meiotic mapping of *smsA*, using several crosses to

markers of linkage group V, located this gene on the right arm of chromosome V, loosely linked and distal to *facA* (and showing $21 \pm 4\%$ recombination with *riboD*; this explains the free recombination observed in crosses to *lysB*, Table 2, *lysB* being located on the left arm of chromosome V).

Only 1 of about 20 analyzed mutations which specifically suppressed MMS sensitivity was found to be nonallelic with *smsA* mutations. This suppressor was isolated in a *proA1* strain and mapped to chromosome III (among 150 haploid segregants from 3 heterozygous diploids 12% unexpected crossovers with markers of chromosome III were found, but >40% recombination to markers on all other chromosomes was observed). This suppressor, *smsB3* (Table 1), abolished MMS sensitivity of *proA2* and *lysB* but not of *phenA*.

Unrelated mutations: Among the sectors selected on MMS media from AA-requiring strains a few were found which also reduced the requirement. Three of these turned out to be cases of revertants, since no AA-requiring segregants were recovered in crosses to standard strains (indicated as "R" in Table 1). In two other cases, suppressors isolated in strains requiring methionine completely abolished the requirement and consequently also the MMS sensitivity (Table 1, *methG* and *methH*). These suppressors were unlinked to *smsA* as well as to the respective *meth* mutations (called *smt* = suppressors of *meth* in Table 1). They were not analyzed further, since many such genes are known to exist (GAJEWSKI and LITWIŃSKA 1968; AYLING 1969; SCOTT and KÄFER 1982).

Effects of *smsA* on the requirement of *pro*: Since the suppressor of MMS-sensitivity selected in *proA1* was not an allele of *smsA*, it was of interest to check effects of *smsA* on *proA1*. Very different results were obtained in crosses to two isolated *smsA* alleles present in strains with different AA mutations, one of them *lysB*, the other *phenA*. While crosses of *proA1* to *phenA*; *smsA2* showed no deviation from the expected unlinked segregation of the three mutations ($P > 0.8$), crosses of *proA1* to *lysB5*; *smsA51* produced none of the expected 25% *pro*⁻ *sms* progeny. Extensive analysis of three different *pro* mutants crossed to two different *lys* and various *smsA* mutations, led to the solution of this puzzling finding. In one of these crosses, *proA2*, a mutant which does not grow at all on MM, showed partial growth in media lacking proline when *smsA* was present. Apparently *smsA* suppressed not only MMS-sensitivity but also the requirement of *proA2* when crossed to *lys*. It therefore seemed likely that for the leaky *proA1* any *smsA* mutation completely suppressed the proline requirement in such crosses. Extensive retests and outcrossing of many potential *proA1*; *smsA* segregants and suitable controls showed that such strains indeed grow in the

absence of proline, but only when lysine is present in test media. It is concluded that *smsA* suppresses the proline requirement of *proA* and *proB* mutations on minimal media supplemented with lysine (shown for *proA1* and *proA2* in Figure 1a; see "MM + Lys" plate). No effects of lysine or phenylalanine were observed for the suppression of MMS-sensitivity on supplemented MM or CM.

Effects of *smsA* on resistance to pfp: It has been known for some time that in *Aspergillus* as well as in *Neurospora*, *lys* strains do not express pfp-resistant mutations which in other strains permit growth on high levels of pfp (KÄFER 1977; KINSEY and STADLER 1969). To test for effects of *smsA* on this unexplained interaction, a *lysF;smsA* strain was crossed to *fpaA proA11* (the complete genotype of this cross was *riboA fpa⁺ pro⁺ lysF;smsA* × *ribo⁺ fpaA proA lys⁺;sms⁺*). In progeny from such a cross the presence of *fpaA* can be assumed when *riboA* is absent, because recombination between *fpaA* and *riboA* is very rare (0.2%) (CLUTTERBUCK 1984). Tests of *fpaA* segregants showed that *smsA* clearly modified growth of *fpaA proA* types on pfp media, while *fpaA lysF* recombinants were barely affected. Namely, *fpaA proA* segregants grew very poorly on CM containing pfp when *smsA* was present, while, as expected, in the absence of *smsA*, all *fpaA proA* types showed the normal high resistance of the *fpaA proA* parent (among 84 tested progeny, 21 were *proA* and *ribo⁺*, i.e., presumably *fpaA proA*; of these, 12 were *sms⁺* and normally pfp resistant, while nine were *smsA* and grew very poorly on pfp media).

Effects of *smsA* on *nicB*: In *Aspergillus*, the mutant *nicB8* which can grow on nicotinic acid or tryptophan is affected by the presence of *phenA*. Double-mutant strains *phenA;nicB* grow considerably on media lacking Nic (i.e., MM + Phen) while on the same medium *nicB* single-mutant strains do not grow (when incubated 2–3 days at 37°). In crosses heterozygous for *smsA* it was found that half of the *phenA;nicB* segregants grew partially as expected, while the other half did not grow in the absence of Nic and the latter all carried *smsA* (Figure 1b). This indicates that *smsA* counteracts the effect of *phenA* on *nicB* (on MM + Phen).

DISCUSSION

The accumulated evidence from crosses of many AA-requiring mutations, plus the tests of all other available mutants of this type, convincingly demonstrates that hypersensitivity to MMS is a general property of AA mutants in *Aspergillus nidulans*. This was confirmed by the direct isolation of AA requirers among induced MMS-sensitive mutants and by the recovery of MMS-sensitive segregants from the back cross of the only observed exception (*lysD20*) which turned out to contain an unlinked suppressor. This

MMS sensitivity was not reversed by high concentrations of the required AA in the MMS media and could also be demonstrated when conidia were treated with MMS for short periods in buffer (results similar to those of Figure 2). While increased uptake of MMS by AA mutants could explain the observed hypersensitivity, these findings and various other considerations make this explanation unlikely (direct measurements of uptake have therefore not been attempted). A major argument for intracellular effects of MMS comes from the identification of a suppressor gene, *smsA*, and many mutant isolates which eliminate the hypersensitivity but not the requirement of any AA mutation. This effect of a single gene on all pathways opens up the question whether *smsA* is related to any of the genes involved in cross-pathway control for enzymes of AA biosynthesis as identified in *Neurospora* (CARSIOTIS, JONES and WESSELING 1974; BARTHELMESS 1984), in yeast (NIEDERBERGER, MIOZZARI and HUTTER 1981; HINNEBUSCH and FINK 1983) and also in *Aspergillus* (PIOTROWSKA 1980). Sensitivity to MMS might then be the result of alkylation of some crucial factor required for regulation in normal but not *smsA* cells (possibly GTP, or a regulatory peptide, since translational regulation seems a possibility) (FINK 1986; TSAMARIAS, ALEXANDRAKI and THIREOS 1986). At this point such explanations are, however, pure speculations and MMS sensitivity of AA-requiring strains as found in *Aspergillus* is no easier to understand than the high sensitivity to histidine of many MMS-sensitive mutants found uniquely in *Neurospora* (NEWMAYER, SCHROEDER and GALEAZZI 1978; NEWMAYER 1986).

The observations of specific effects of *smsA* on growth of *pro* and *nicB* mutants (under special circumstances) do not provide any useful hints or explanations. However, they demonstrate clearly that *smsA* has effects in the absence of MMS. The curious finding that strains carrying *smsA* and *proA1* or *proB* grow almost like wild type on MM + lysine may be related to the specific effect of *smsB*. This suppressor, isolated in a *proA1* strain, resembles *smsA* and suppresses MMS sensitivity of *pro* and *lys* mutants, but not of *phenA*. A special relationship of *pro*, *lys* and *smsA* is further indicated by the observation that, when *smsA* is present, *fpaA proA* double mutant strains become sensitive to pfp and resemble *fpaA lys* strains which do not express the pfp-resistance mutation.

I am indebted to MARIE BÉDARD and MARY-ANN DOBROVOLNY for diligent assistance in the genetic analysis of many crosses. I also appreciate the excellent technical assistance of ANNE TITTLER and the expert preparation of diagrams and photographs by DOT LUK, ROBERT LAMARCHE and GUY L'HEUREUX. This work was supported by operating grant A 2564 from the National Science and Engineering Research Council of Canada.

LITERATURE CITED

- AYLING, P. D., 1969 Methionine suppressors in *Aspergillus nidulans*: their genetics and behaviour in heterokaryons and diploids. *Genet. Res.* **14**: 275–289.
- BARRATT, R. W., G. B. JOHNSON and W. N. OGATA, 1965 Wild-type and mutant stocks of *Aspergillus nidulans*. *Genetics* **52**: 233–246.
- BARTHELMESS, I. B., 1984 A lethal allele at the putative regulatory locus, *cpc-1*, of cross-pathway control in *Neurospora crassa*. *Mol. Gen. Genet.* **194**: 318–321.
- BORAM, W. R. and H. ROMAN, 1976 Recombination in *Saccharomyces cerevisiae*: a DNA repair mutation associated with elevated mitotic gene conversion. *Proc. Natl. Acad. Sci. USA* **73**: 2828–2832.
- CARSIOTIS, M., R. F. JONES and A. C. WESSELING, 1974 Cross-pathway regulation: histidine-mediated control of histidine, tryptophan and arginine biosynthetic enzymes in *Neurospora crassa*. *J. Bacteriol.* **119**: 893–898.
- CLUTTERBUCK, A. J., 1984 Loci and linkage map of the filamentous fungus *Aspergillus nidulans* (Eidam) Winter ($n = 8$). pp. 265–273. In: *Genetic Maps*, Vol. 3, Edited by S. J. O'Brien. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- FINK, G. R., 1986 Translational control of transcription in eukaryotes. *Cell* **45**: 155–156.
- GAJEWSKI, W. and J. LITWIŃSKA, 1968 Methionine loci and their suppressors in *Aspergillus nidulans*. *Mol. Gen. Genet.* **102**: 210–220.
- HASTIE, A. C., 1970 Benlate-induced instability in *Aspergillus* diploids. *Nature* **226**: 771.
- HINNEBUSCH, A. G. and G. R. FINK, 1983 Positive regulation in the general amino acid control of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **80**: 5374–5378.
- KÄFER, E., 1958 An 8-chromosome map of *Aspergillus nidulans*. *Adv. Genet.* **9**: 105–145.
- KÄFER, E., 1977 Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. *Adv. Genet.* **19**: 33–131.
- KÄFER, E. and O. MAYOR, 1986 Genetic analysis of DNA repair in *Aspergillus*: evidence for different types of MMS-sensitive hyperrec mutants. *Mutat. Res.* **161**: 119–134.
- KÄFER, E. and E. PERLMUTTER, 1980 Isolation and genetic analysis of MMS-sensitive *mus* mutants of *Neurospora*. *Can. J. Genet. Cytol.* **22**: 535–552.
- KINSEY, J. A. and D. R. STADLER, 1969 Interaction between analogue resistance and amino acid auxotrophy in *Neurospora*. *J. Bacteriol.* **97**: 1114–1117.
- MEADE, J. H. and T. R. MANNEY, 1983 Sensitivity of tryptophan, tyrosine and phenylalanine mutants of *Saccharomyces cerevisiae* to phenethyl alcohol. *Genetics* **104**: 235–240.
- NEWMAYER, D., 1984 *Neurospora* mutants sensitive both to mutagens and to histidine. *Curr. Genet.* **9**: 65–74.
- NEWMAYER, D., A. L. SCHROEDER and D. R. GALEAZZI, 1978 An apparent connection between histidine, recombination, and repair in *Neurospora*. *Genetics* **89**: 271–279.
- NIEDERBERGER, P., G. MIOZZARI and R. HÜTTER, 1981 Biological role of the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **1**: 584–593.
- PIOTROWSKA, M., 1980 Cross-pathway regulation of ornithine carbamoyltransferase synthesis in *Aspergillus nidulans*. *J. Gen. Microbiol.* **116**: 335–339.
- PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD and A. W. J. BUFTON, 1953 The genetics of *Aspergillus nidulans*. *Adv. Genet.* **5**: 141–238.
- PRAKASH, S., L. PRAKASH, W. BURKE and B. A. MONTELEONE, 1980 Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. *Genetics* **94**: 31–50.
- SCOTT, B. R. and E. KÄFER, 1982 *Aspergillus nidulans*—an organism for detecting a range of genetic damage. pp. 447–479. In: *Chemical Mutagens*, Vol. 7, Edited by F. J. DE SERRES and A. HOLLAENDER. Plenum, New York.
- TZAMARIAS, D., D. ALEXANDRAKI and G. THIREOS, 1986 Multiple *cis*-acting elements modulate the translational efficiency of GCN4 mRNA in yeast. *Proc. Natl. Acad. Sci. USA* **83**: 4849–4853.

Communicating editor: R. L. METZENBERG